

The RNA modification database, RNAMDB: 2011 update

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ABSTRACT

Since its inception in 1994, *The RNA Modification Database* (RNAMDB, <http://rna-mdb.cas.albany.edu/RNAmods/>) has served as a focal point for information pertaining to naturally occurring RNA modifications. In its current state, the database employs an easy-to-use, searchable interface for obtaining detailed data on the 109 currently known RNA modifications. Each entry provides the chemical structure, common name and symbol, elemental composition and mass, CA registry numbers and index name, phylogenetic source, type of RNA species in which it is found, and references to the first reported structure determination and synthesis. Though newly transferred in its entirety to The RNA Institute, the RNAMDB continues to grow with two notable additions, agmatidine and 8-methyladenosine, appended in the last year. *The RNA Modification Database* is staying up-to-date with significant improvements being prepared for inclusion within the next year and the following year. The expanded future role of *The RNA Modification Database* will be to serve as a primary information portal for researchers across the entire spectrum of RNA-related research.

INTRODUCTION

The chemical composition of an RNA molecule allows for its inherent ability to play many roles within biological systems. This ability is further enhanced through the site selected addition of the 109 currently known post-transcriptional modifications catalyzed by specific RNA modification enzymes (1). These naturally-occurring modifications are found in all three major RNA species (tRNA, mRNA and rRNA) in all three primary phylogenetic domains (Archaea, Bacteria and Eukarya) as well as in a handful of other RNA species such as snRNA (2–5) (Table 1). The modifications are one of the most evolutionarily conserved properties of RNAs. Due in large part to comprehensive investigations into the structural and functional roles of modified nucleotides in tRNA, significant advancements have been achieved in our understanding of the various roles played by these modifications (2,6–9). The need to provide a comprehensive, searchable database to house this wealth of knowledge led to the first iteration of *The RNA Modification Database* (RNAMDB) in 1994 (10). The current version of the database, now housed at The RNA Institute at the University at Albany SUNY, contains all naturally-occurring, RNA-derived modified ribonucleosides for which the chemical structures are known. The RNAMDB provides a user-friendly, searchable interface that directs the user to a detailed information page for each database entry (Figure 1).

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Users are invited to submit comments regarding existing entries, including errors and omissions, as well as suggestions for improvements to the following email address: rnamdb@albany.edu.

DATABASE AND CONTENTS

A comprehensive introduction page provides a detailed overview of the current state of the RNAMDB, an

Table 1. The RNA modifications in the database are present in all phylogenetic groups and in many of the known RNA species

RNA	Phylogenetic source		
	Archaea	Bacteria	Eukarya
tRNA	43	45	51
rRNA		1 ^a	4 ^a
SSU	11	8	18
LSU	8	15	12
5S	3		1
5.8S			5
mRNA			13
tmRNA		2	
snRNA			11
Chromosomal RNA			2
Other Small RNA			1

^aDenotes rRNA modifications in which the subunit of origin is either unknown or reported as a mixture of subunits.

in-depth description of the entries and a page specifically dedicated to modifications found in ribosomal RNA. The overview section of the introduction page provides a focus on commonly used symbols representing RNA modifications, reasons for exclusion of certain modified nucleosides and an extensive listing of reviews for further reference.

Presently, the information page for each RNAMDB entry contains the following details (Figure 2a–g).

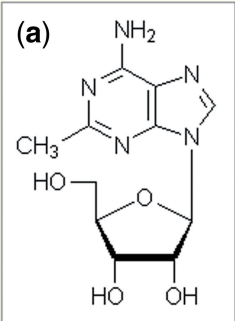
- Chemical structure of the modified nucleoside: this structure is simplistic and is not meant to imply the preferred orientation of dihedral angles or sugar pucker.
- Common name and symbol.
- The searchable Chemical Abstracts registry numbers and index name, which may provide stereochemical information not shown in the chemical structure.
- Elemental composition and molecular weight.
- Phylogenetic source of the RNA (i.e. Archaea, Bacteria or Eukarya) and the type of RNA species in which it is found (i.e. tRNA, rRNA, etc.).
- Literature references for the first reported structure and synthesis. Reports of more recent synthesis or refinements can be found using the Chemical Abstracts registry numbers noted above.
- Comments pertaining to any of the information provided on the entry information page provided by database curators.

Figure 1. The user-friendly search page makes it easy for users to find RNA modifications in the database based on a variety of different criteria.

The RNA Modification Database

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2-methyladenosine

(a) 

(b) **common name:**
2-methyladenosine

symbol:
m²A

CA index name:
Adenosine, 2-methyl-

CA registry numbers:
ribonucleoside 16526-56-0
base 1445-08-5

elemental composition:
C₁₁H₁₅N₅O₄

(d) **nucleoside mass:**
281.27

(e)

RNA	phylogenetic source		
	archaea	bacteria	eukarya
tRNA	+	+	
rRNA		23S [1] 23S [2]	

(f) **Structure:** [3]

Synthesis: [4]

(g) **Comment:** Although ref [1] reports m²A to be in a mixture of SSU and LSU subunits, various more recent studies have shown that m²A is not present in the bacterial SSU (see [SSU database](#)) but is in the LSU [5].

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[002]

Figure 2. The entry information page for 2-methyladenosine shows a typical representation of the statistics that are contained within the database.

An example of a single entry information page for 2-methyladenosine has been provided (Figure 2). The RNAMDB contains 109 such entries that consist of 93 found in tRNAs, 31 in rRNA, 13 in mRNA and 14 in other RNA species such as snRNA, snoRNA and miRNA (3,4,6) (Table 1).

In addition to the Introduction and Search pages, the website contains a compilation of links to other helpful databases and search tools, and provides access to a suite of programs, Masspec Toolbox, designed to assist in the identification and characterization of nucleic acids by mass spectrometry (MS). These dedicated programs are capable of calculating the mass of ribo- and deoxyribo-oligonucleotides from their sequence, as well as predicting the masses of products obtained by submitting these molecules to tandem mass spectrometry (MS/MS), or

to digestion by endo- and exonucleases. Other tools can calculate nucleotide and elemental composition from a certain mass and provide the corresponding isotopic distribution. All of these tools allow for users to account for the presence of chemically modified nucleotide residues.

RECENT ADDITIONS

Since the publication of the last database overview (3), 14 new entries have been added to the RNAMDB, including agmatidine and 8-methyladenosine, which have both been added within the past year (11–25) (Table 2). Agmatidine (C⁺) was recently found in archaeal AUA-decoding tRNA^{Ile2}, where it performs a function similar to lysidine, a modified cytidine residue containing lysine in

Table 2. Fourteen modifications have been added to the database since the last update was published in 1999

Entry	Symbol	Common name	Phylogenetic source	RNA type	References
96	m ¹ Gm	1,2'- <i>O</i> -dimethylguanosine	Archaea	tRNA	(11,12)
97	m ¹ Am	1,2'- <i>O</i> -dimethyladenosine	Eukarya	tRNA	(13,14)
98	tm ⁵ U	5-taurinomethyluridine	Eukarya	tRNA	(15,16)
99	tm ⁵ s ² U	5-taurinomethyl-2-thiouridine	Eukarya	tRNA	(15,16)
100	imG-14	4-demethylwyosine	Archaea	tRNA	(17,18)
101	imG2	isowyosine	Archaea	tRNA	(17)
102	ac ⁶ A	N ⁶ -acetyladenosine	Archaea	tRNA	(19,20)
103	inm ⁵ U	5-(isopentenylaminomethyl)uridine	Bacteria	tRNA	(21)
104	inm ⁵ s ² U	5-(isopentenylaminomethyl)-2-thiouridine	Bacteria	tRNA	(21)
105	inm ⁵ Um	5-(isopentenylaminomethyl)-2'- <i>O</i> -methyluridine	Bacteria	tRNA	(21)
106	m ^{2,7} Gm	N ² ,7,2'- <i>O</i> -trimethylguanosine	Archaea	tRNA	(21)
107	m ⁴ ,2Cm	N ⁴ ,N ⁴ ,2'- <i>O</i> -trimethylcytidine	Archaea	tRNA	(22)
108	C ⁺	agmatidine	Archaea	tRNA	(23)
109	m ⁸ A	8-methyladenosine	Bacteria	rRNA	(24,25)

place of the C2-oxo group, in bacteria (23). In *Escherichia coli*, the presence of lysidine at the first position of the anticodon allows for AUA-decoding tRNA^{Ile2} to recognize A and not G in the third position of the codon (26). Agmatidine has a structure very similar to lysidine in which agmatine (decarboxy-arginine) replaces the C2-oxo group of cytidine (Figure 3a) and, at the first position of the anticodon, performs the same function of differentiating between A and G at the third position of the codon in *Haloarcula marismortui* (23). The discrimination between A and G that results from the presence of lysidine or agmatidine is hypothesized to play an important role in the stabilization of potential positively charged tautomeric structures at neutral pH, which are able to selectively base pair with A (23).

Similar to 2-methyladenosine (m²A) (27), 8-methyladenosine (m⁸A, Figure 3b) is present in the large subunit of bacterial ribosomes, specifically in the peptidyl transferase center at position A2503 of 23S rRNA as confirmed by comparison to the chemically synthesized mononucleoside (24). Whereas the m²A chemistry shows only a small amount of antibiotic resistance (28), the m⁸A moiety confers extensive resistance to five major classes of peptidyl transferase-targeting antibiotics (29). Interestingly, the m⁸A mononucleoside was also synthesized in 1993 and shown to be a potent inhibitor of vaccinia virus (25). This modification is a clear example of why it is important to understand the purpose of RNA modifications in living systems and how they can be utilized for therapeutic purposes. As an interesting side note, the presence of dimethylated A2503 (m²m⁸A) was also reported (24); however, this modification has yet to be fully characterized or structurally confirmed.

THE RNA INSTITUTE TAKES OVER AS CURATOR

As of January 2009, upkeep and supervision of the RNAMDB has been turned over to The RNA Institute. Already, this transfer has resulted in a major layout makeover in May 2009 to match the theme of the newly established institute. As a part of its mission, The RNA

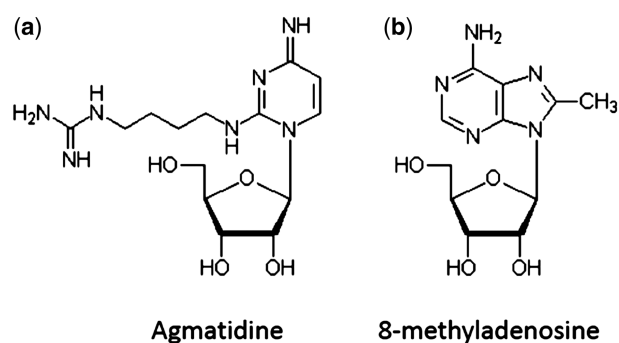


Figure 3. The two most recent additions to The RNA Modification Database: (a) agmatidine and (b) 8-methyladenosine, have been discovered and structurally characterized within the past year.

Institute will be expected to become a proposed hub of cutting edge RNA research and a leading provider of structural and functional information in the field of RNA modifications. For this reason, The RNA Institute will provide the logical base for new enhancements aimed at expanding the focus of the current infrastructure to cover other important aspects of modern RNA research. These enhancements will be embodied by the proposed creation of a portal that will constitute a primary resource for researchers across the entire spectrum of RNA-related disciplines. The portal will not only include the current RNAMDB and associated tools, but from this new infrastructure, users will be able to access other informatics resources for RNA science, including different repositories of experimental protocols, force field parameters for molecular dynamics simulations (30), and sister databases dedicated to non-natural RNA modifications. The infrastructure for all of the above-mentioned improvements will be implemented on a rolling basis as they are completed during 2011. A full-featured RNA portal is a goal to be reached in 2012.

The experimental protocols made available by the RNAMDB portal will comprise established procedures that are common practices in a typical RNA lab, but will also include new cutting-edge methods developed by the broader RNA community. The RNA Institute will be

expected to play an important role in populating this section, due to the research interests of its members and the nature of their collaborations, which involve the development of enabling technologies based on a wide range of experimental approaches, including nuclear magnetic resonance, X-ray crystallography, single-molecule spectroscopy and, with the recent opening of The RNA Mass Spectrometry Center, high-resolution mass spectrometry. However, any investigator engaged in RNA research will be encouraged to share his/her favorite techniques with the broader RNA community through the portal. Any new protocol will require proof, either through publication in a peer-reviewed journal or empirical representative data that demonstrate its usefulness, effectiveness and reproducibility. Links to other repositories of information on specific methods, such as peer-reviewed scientific journals, will allow users to conveniently access the most up-to-date and well-characterized methods in the literature. While it is important to note that this enhancement will take a great deal of time to populate in the next 2 years with useful protocols from the various disciplines within the RNA community, the initial steps for incorporation into the RNAMDB are currently in progress.

The proposed portal will be hosting a number of tools for predicting RNA secondary structure folding, the energy of those structures, and their predicted biophysical properties, such as melting temperature. For instance, the popular RNA folding programs Mfold (31) and UNAFold (32) developed by Dr Michael Zuker will soon be accessible through the current RNAMDB infrastructure and will be subsequently incorporated in the portal. Full integration will be achieved by making the information stored in the RNAMDB directly accessible by the folding algorithms in such a way as to enable users to calculate the possible effects of RNA modifications on energetics and structure stability. Since a drastic restructuring of the source code will be required, full integration of Dr Zuker's folding programs will likely be implemented during the 2012–13 academic year; however, the programs are currently included, in their previous state, as a part of the RNAMDB. In a similar fashion, we plan on modifying existing software to enable the productive utilization of the information in the RNAMDB. For example, full integration with the programs in the existing Masspec Toolbox suite will greatly benefit the MS identification and characterization of natural RNAs extracted from living organisms. The new tools developed for the portal will be designed to take advantage of the information stored in the site and, thus, will be capable of accessing the different types of data and seamlessly communicating with one another across the board.

The proposed portal will include a constantly updated compilation of hyperlinks to external sources that may provide additional information for the identification and the structure–function investigation of RNA. In addition to repositories of experimental protocols, these links will direct users to pertinent peer-reviewed journals, the *RNA Society*, the *Transfer RNA Database* (33), the *Collaboratory for MS3D* (34) and many others. Links

will also smoothly point users to the external primary sources of information contained in the RNAMDB website. For instance, users will no longer be required to copy and paste the Chemical Abstracts registry numbers from the RNAMDB to the CAS Registry website. Instead, they will simply need to click on the hyperlinked CAS Registry number. Similarly, all references to both internal and external sources will be hyperlinked to provide quick access to all primary information. Additionally, it has become apparent that the information contained within the RNAMDB can be supplemented by cross-referencing with other relevant information residing in other databases such as *Modomics* (1) and *tRNAdb* (33).

Other planned enhancements to the RNAMDB functionalities include incorporation and cross-referencing with new sister databases containing the growing amount of information on both natural and non-natural RNA modifications. A sister database of force field parameters for molecular dynamics simulations will also be included as a supplement to the newly incorporated table of modified nucleoside base pairing free energy (ΔG) minima (35). Although excluded from the current RNAMDB, non-natural modifications are gaining increasing interest in both academic and corporate settings. These types of modifications include those used as research tools because of their ability to fluoresce (36), to restrict certain geometries, including backbone alterations (37), to enable unusual base pairing schemes (38) and to act as tools for probing structure (39,40). Furthermore, other non-natural nucleosides that have been discovered as the products of interactions with pharmaceuticals and as a result of environmental stimuli will also be included.

Finally, it is becoming apparent that a more modern user interface will be needed to accommodate these new enhancements. With recent technological improvements, such as deep transcriptome analysis (41) and high-throughput mass spectrometry (42,43), which have led to advancements in our current understanding of RNA in biological systems, it has become necessary to create a more automated method for researchers to submit their findings to the RNAMDB for consideration. The new user interface is being developed for implementation during 2011 and will seek to create a more user-friendly experience while adding improved functionality. The new interface will allow the curators to dedicate more of their time and resources towards validating entries and developing new features. In this direction, while past iterations allowed for unpublished modifications to be included (Table 2), the new implementation and curating structure will require publication as a criterion for entry into the RNAMDB. This will create a benchmark for the inclusion of modifications that have been diligently characterized and will reduce the likelihood of inaccurate entries.

AVAILABILITY

The RNA Modification Database is freely available to researchers via the web address: <http://rna-mdb.cas>

.albany.edu/RNAMods/. Additionally, the programs contained in the Masspec Toolbox are also free for public use through the link on the database homepage and via the web address: <http://rna-mdb.cas.albany.edu/RNAMods/rnamass.htm>. Users of information contained within this database are requested to cite this article as their source of information.

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